SYNTHESIS OF D_3-METOCLOPRAMIDE

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SUMMARY

Metoclopramide-d_3 with the three deuterium atoms in the methoxy function ortho to the benzamide group was synthesized. A previously published synthesis was followed but was extensively modified in the final steps to increase overall yield. Structure was verified by NMR and GC-MS.

KEYWORDS

Benzamide,

labelled compound,

stable isotope,

metoclopramide,

4-Amino-5-chloro-N-[(diethylamino)ethyl]-2-trideuteromethoxybenzamide.

INTRODUCTION

Metoclopramide, 4-Amino-5-chloro-N-[(diethylamino)ethyl]-2-methoxybenzamide, I, (Figure I), is an anti-emetic commonly used world-wide in human therapy [1,2].

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The pharmacokinetic parameters and metabolic transformations (in animals and man) of metoclopramide had been determined and published by numerous investigators though the results do not seem completely superimposable [3-10].

All the analytical methods published thus far have used a related substance, such as bromopride III (Figure I), for the internal standard in the analytical procedure [10].

To improve the precision, accuracy and rapidity of our quantitative GC/MS method we decided to synthesize D_3-metoclopramide, 4-Amino-5-chloro-N-[(diethylamino)ethyl]-2-trideuteromethoxybenzamide, II (Figure I), to be used as the internal standard. D_3-metoclopramide is also being used in the stable isotope method of determining metabolic transformations both in animals and man.

We therefore report the complete synthesis of D_3-metoclopramide.

To improve the yield we used a variation of the method developed by SESIF [11] illustrated in Figure II.

In our present work we have essentially modified only the order in which the reactions are performed in order to increase the yield of the D_3-analog. Our reaction schemes are presented in Figures III and IV. Represented in Figure II are the syntheses which are common to both the natural and the deuterated analog. Presented in Figure IV are the reactions in which the natural and the deuterated analogs differ.

MATERIALS AND METHODS

All infrared spectra were recorded on a Beckman Model IR4230 infrared spectrophotometer.

All ^{1}H-Nuclear Magnetic Resonance Spectra were recorded on a Perkin-Elmer Model R-12 60 Megahertz NMR spectrometer.

All melting points are uncorrected and were determined on a

Mettler FP61 instrument.

A quadrupole R10-10 Mass spectrometer (Nermag, Rueil-Malmaison, France) coupled through a jet separator to a Girdel Series 31 gas chromatograph (Delsi, Suresnes, France) was used. The chromatograph was fitted with a 2.1 meter glass column packed with 3 % OV-1 on Chromosorb WAW - DMCS.

The temperatures were 290°C for both the injector and the interface. The ion source temperature was maintained at 100°C with a pressure of 2 x 10^{-4} torr for the ionizing gas (NH_3). The ionizing current was 230 μA and the ionizing voltage was 70 eV. Mass spectra were recorded at a scan speed of 2 ms/a.m.u. with unit resolution.

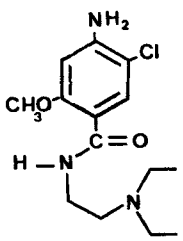
Methelute^R (trimethyl anilinium hydroxide) and pentafluoropropionic acid anhydride were purchased from Pierce Chemical Company (Rockford, ILLINOIS , U.S.A.).

All other reagents and solvents used, either of synthetic or analytical grade, were purchased either from Merck Chemical Company or Aldrich Chemical Company.

Authentic metoclopramide was obtained as a gift from Laboratoires Delagrange (Paris, France).

REACTION (1) - Preparation of 4-Amino-2-hydroxybenzoic acid methylester, IX.

30.6 g. (0.2 M) of 4-Amino-2-hydroxybenzoic acid, VIII, dissolved in 81 ml of methanol containing 20 ml of concentrated H_2SO_4, were heated at reflux for 8 hours. The mixture was then evaporated to dryness under reduced pressure. The resulting crystalline residue was dissolved in 500 ml of 10% aqueous solution of potassium bicarbonate and subsequently extracted with dichloromethane. The organic phase was dried over sodium sulfate and the organic solvent eliminated under



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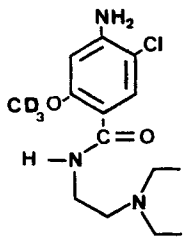

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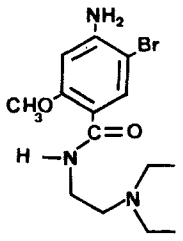

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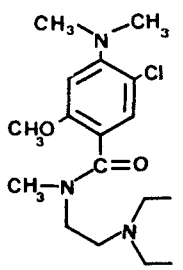

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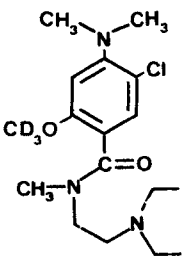

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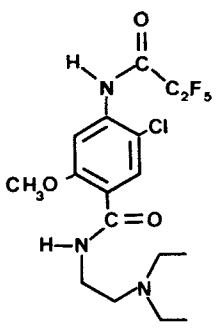

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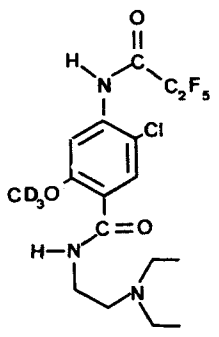
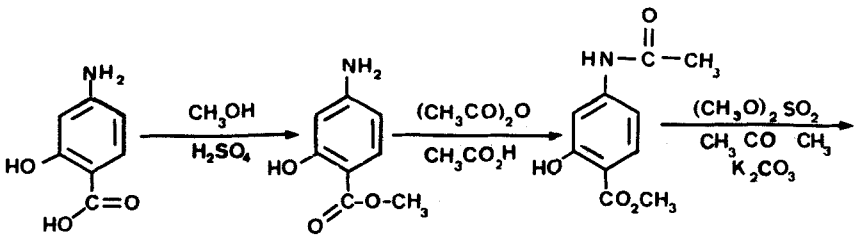
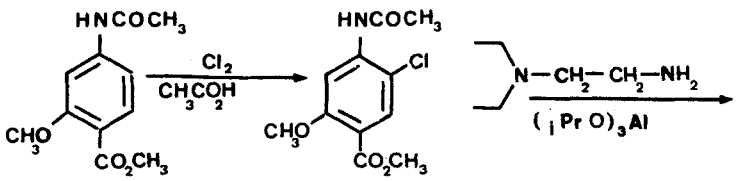


VII

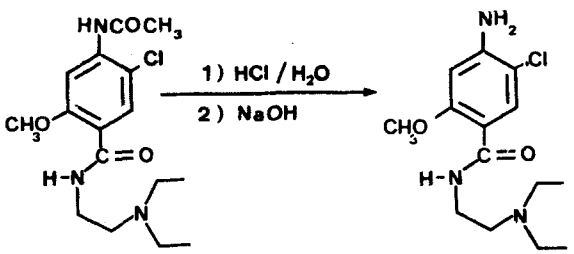
FIGURE I : Metoclopramide, I, D_3-Metoclopramide, II, Bromopride, III, Trimethyl Metoclopramide, IV, Trimethyl D_3-Metoclopramide, V, N-Pentafluoropropionyl Metoclopramide, VI, and N-Pentafluoropropionyl D_3-Metoclopramide, VII.



VIII IX X

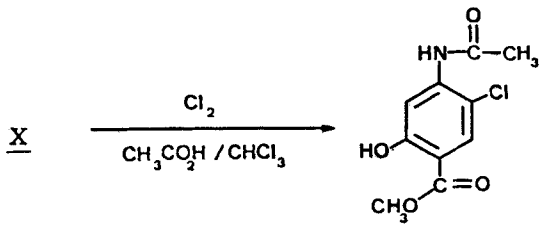


XI XII a



XIII I

FIGURE II : Previous Synthesis of Metoclopramide, I.



XIV

FIGURE III : First-half of Synthesis of Metoclopramide, I, and D_3-Metoclopramide, II.

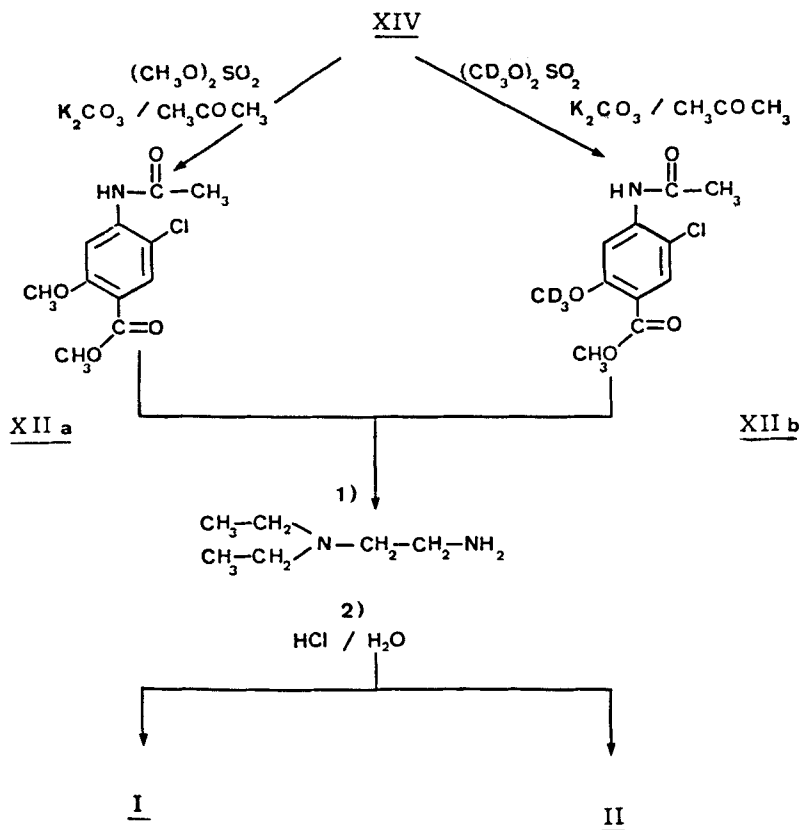


FIGURE IV : Second-half of synthesis of Metoclopramide, I, and D_3-Metoclopramide, II.

reduced pressure. The 27.6 g. of crystalline powder of IX obtained had a melting point of 119.5°C and corresponded to an 81 % yield. The product in a KBr pellet had characteristic infrared bands for OH(3475 cm^{-1}), NH_2(3245, 3380 and 1280 cm^{-1}) and CO(1715 cm^{-1}). The ^{1}H-NMR spectrum in $CDCl_3$ yielded a singlet (3 protons) at 3.85 ppm, a multiplet (4 protons) at 6.15 ppm, a singlet (1 proton) at 7.65 ppm and a singlet (1 proton) at 11.0 ppm.

REACTION (2) - Preparation of 4-(Acetylamino)-2-hydroxybenzoic acid methylester, X.

20 g. (0.13 M) of the ester IX were dissolved in a minimum volume of absolute ethanol or glacial acetic acid. 11.5 ml of acetic anhydride were added and stirred continuously for 1 hour. The solvents were then evaporated under reduced pressure. The resulting residue was disssolved in about 25 ml of 1 N NaOH, precipitated by 1 N HCl, extracted by chloroform or dichloromethane, dried over magnesium sulfate and evaporated under reduced pressure. The 23 g. of crystalline powder of X obtained had a melting point of 152°C and corresponded to a 94 % yield. The product in a KBr pellet had characteristic infrared bands for OH(3400 cm^{-1}), CO(1700 and 1650 cm^{-1}) and $COO\underline{CH_3}$(1250 cm^{-1}). The ^{1}H-NMR spectrum in $CDCl_3$ had 3 doublets (1 proton each) at 7.2 ppm (J_1=3 Hz, J_2= 12 Hz), 7.5 ppm (J=3 Hz) and 7.85 ppm (J=12 Hz). There were also 2 singlets (1 proton each) for the NH proton at 10.0 ppm and the OH proton at 10.9 ppm and two other singlets 3 protons at 2.2 ppm (CH_3-CO) and 4.0 ppm (3 protons CH_3-O).

REACTION (3) - Preparation of 4-(Acetylamino)-5-chloro-2-hydroxybenzoic acid methylester, XIV.

20.9 g. (0.1 M) of the amide X were dissolved in 300 ml of a mixture of dichloromethane and crystallizable acetic acid (1:1, v/v).

This mixture was cooled to -10°C by a mixture of ice, water and salt. Chlorine gas was bubbled through the mixture until the weight increased by 3.55 g. and the mixture allowed to return to ambient temperature during one hour while being agitated. The mixture was then alkalinized with potassium carbonate and extracted with dichloromethane. The organic phase was dried over magnesium sulfate and evaporated under reduced pressure. The 16 g. of a crystalline powder of XIV obtained after recrystallization in absolute ethanol corresponded to a 63 % yield and had a melting point of 170°C. The product in a KBr pellet had characteristic infrared bands for OH(3350 cm^{-1}), NH(3280 cm^{-1}), $CO_{(ester)}$ (1700 cm^{-1}), $CO_{(amide)}$ (1650 cm^{-1}), and C=C(1590 cm^{-1}). The ^{1}H-NMR spectrum in $CDCl_3$ had at 2.2 ppm a singlet of 3 protons (CH_3 amide), at 3.9 ppm a singlet of 3 protons (CH_3-ester), at 7.8 ppm a singlet of 1 proton (aromatic), a broadened peak of 1 proton (NH) centered at 8.10 ppm, a sharp singlet of 1 proton (aromatic) at 8.10 ppm and at 10.65 ppm a singlet of 1 proton (OH).

REACTION (4a) AND (4b) - Preparation of 4-(Acetylamino)-5-chloro-2-methoxybenzoic acid methylester, XIIa, and 4-(Acetylamino)-5-chloro-2-trideuteromethoxybenzoic acid methylester, XIIb.

16 g (0.068 moles) of phenol XIV were dissolved in 160 ml acetone, 3.4 g (0.075 moles) of potassium carbonate were added along with 0.068 moles of dimethylsulfate (4a) or di-(trideutero)methyl sulfate (4b). The mixture was refluxed for 6 hours at 60°. 50 % potassium carbonate and 10 % dimethyl sulfate were then added and refluxed for 4 hours. The solvent was removed in vacuo and the residue alkalinized with 1 N sodium hydroxide and extracted twice with dichloromethane. The organic phase was dried over magnesium sulfate and the dichloromethane removed in vacuo. The products XIIa and XIIb were crystallized in toluene and yielded 14.2 g (80 %) methyl and 14.15 g (80 %) trideuteromethyl respectively. A melting point of 151-153°C was found for both products.

The products in KBr pellets had characteristic infrared bands at : NH(3410 cm^{-1} and 1570 cm^{-1}), CO ester (1710 cm^{-1}) CO amide (1681 cm^{-1}) C=C(1600 cm^{-1}) CH(3000-2800 cm^{-1}) for both VIIa and VIIb and CD(2040 cm^{-1}) for VIIb. The ^{1}H-NMR spectra in $CDCl_3$ had a singlet of 3 protons at 2.2 ppm (CH_3 CO-N) for both VIIa and VIIb. A singlet of 6 protons at 3.9 ppm was assigned to CH_3-O and CH_3-OCO for VIIa and 3 protons (CH_3-OCO) for VIIb. A sharp singlet on a broadened peak integrating for 2 protons total at 7.8 ppm for the aromatic and NH respectively was found. A singlet of 1 proton appeared at 8.25 ppm (aromatic).

REACTIONS (5a) AND (5b) - Preparation of 4-Amino-5-chloro-N-[(diethylamino)ethyl]-2-methoxybenzamide, I, and 4-Amino-5-chloro-N-[(diethylamino)ethyl]-2-trideuteromethoxybenzamide, II.

13 g (0.05 mole) of the methylesters XIIa, XIIb were heated at 60° in 28 ml (0.20 mole) of N,N-diethylaminoethylamine for 16 hours. The mixture was stirred with diisopropyl ether and 1 N sodium hydroxide (1:1 v:v) for 2 hours and allowed to stay at room temperature for the night. The benzamide crystallized at the interface and was filtered. The residue was refluxed in a mixture of 50 ml hydrochloric acid and 80 ml of water for 1h30. After cooling in ice the solution was alkalinized with potassium bicarbonate, the metoclopramide precipitated and was filtered off. Recrystallization in ethanol after decoloration with active charcoal yielded 10.4 g Ia and 10.5 g Ib as crystalline powders (69 % yield) with melting points of 144°C for both Ia and Ib. The products Ia and Ib in KBr pellets had characteristic infrared bands for NH(3400 cm^{-1}) NH_2(3300 cm^{-1} 3230 cm^{-1}), CH(2980-2800 cm^{-1}), CD(2270-2240-2210-2040 cm^{-1}) for Ib, C=O (1630 cm^{-1}) C=C (1590 cm^{-1}). The ^{1}H-NMR spectrum in $CDCl_3$ had a triplet of 6 protons at 1.05 ppm J=7 Hz, (CH_3-CH_2), a quadruplet and a triplet of 6 protons total at 2.55

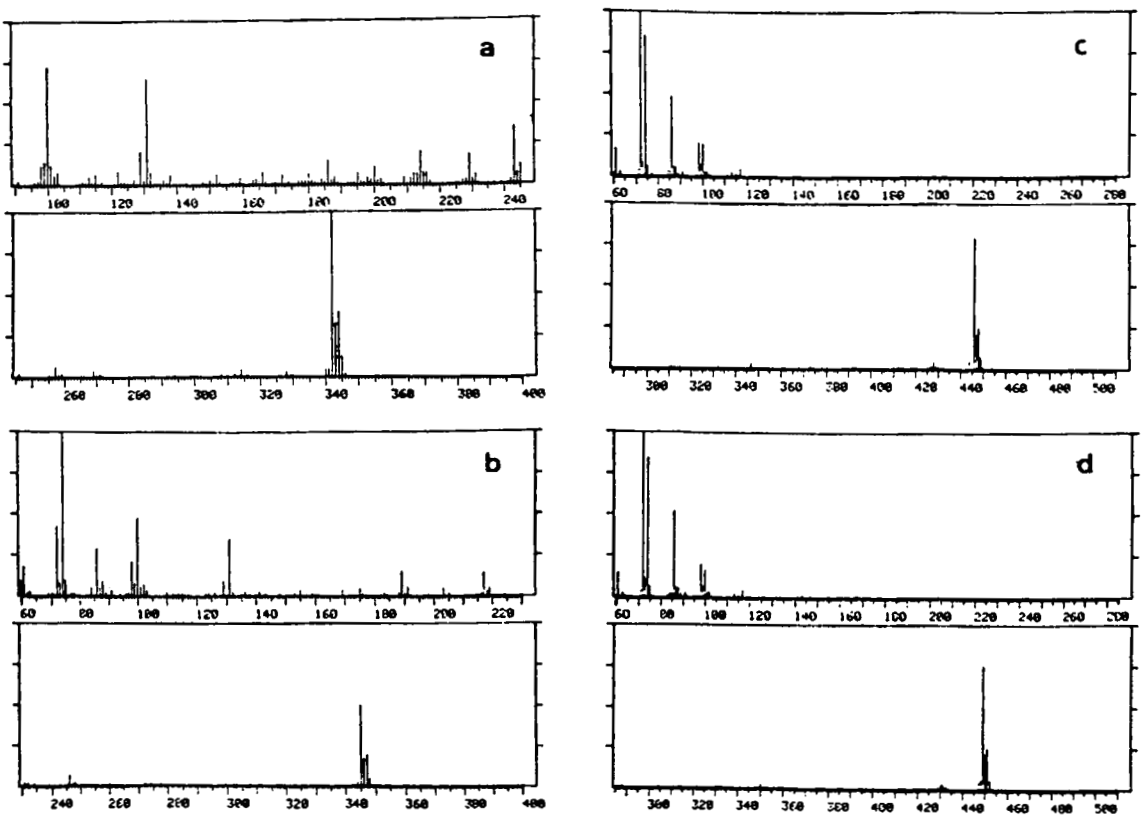


FIGURE V : Chemical Ionization (NH_3) Mass Spectra of Trimethyl Metoclopramide, IV (a), Trimethyl D_3-Metoclopramide, V (b), N-Pentafluropropionyl Metoclopramide, VI (c), and N-Pentafluropropionyl D_3-Metoclopramide, VII (d).

and 2.57 ppm respectively J= 7 Hz assigned to $\underline{CH_2}$-CH_3 and CH_2-$\underline{CH_2}$-N. A triplet of 2 protons at 3.30 ppm, J=7 Hz was assigned to CO-N-$\underline{CH_2}$-CH_2 -N. A singlet of 3 protons appeared at 3.85 ppm for Ia, absent in Ib, was assigned to CH_3O. A broadened peak of 2 protons appeared at 4.55 ppm for the NH_2 group and a singlet of 1 proton at 6.32 ppm for the aromatic proton meta to the benzamide. A singlet of 1 proton appeared at 8.12 ppm for the aromatic proton ortho to the benzamide and a broadened peak of 1 proton appeared at 8.20 ppm for the NH amide.

RESULTS AND DISCUSSION

We were able to synthesize the required deuterium labelled metoclopramide both by using a known synthesis directly and by modifying the reaction order of this synthesis which improved the overall yield. Structural verification was performed by ^{1}H-NMR and IR of both the unlabelled and deuterium labelled products. The disappearance of the unlabelled products characteristic signals in ^{1}H-NMR was due to incorporation of deuterium atoms into the molecule and gave an indication that the synthesis was successful.

To confirm these indications we derivatized both the unlabelled and labelled product with the common reagents trimethyl anilinium hydroxide and pentafluoropropionic anhydride. The mass spectra were recorded under GC-MS (CI-NH_3) conditions and are presented in Figure V. The mass of the quasi-molecular ions and of certain indicative fragments of the labelled product increased by 3 a.m.u. This allowed us both to confirm the identity of the product and to measure the isotopic purity which was determined to be 99.6% labelled product.

The labelled product has proved useful both as the internal standard in GC-MS(CI-NH_3) determinations of the pharmacokinetic parameters of the natural product in man as well as in determinations of the metabolic transformations in animals by co-administration with the natural products. Reports on these works are forthcoming.

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